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APPLICATION NUMBER: *60/590,987*
FILING DATE: *July 26, 2004*
RELATED PCT APPLICATION NUMBER: *PCT/US04/43969*

Certified by

Jon W. Dudas

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16805 U.S. PTO

072604

PROVISIONAL APPLICATION COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION Under 37 CFR 1.53 (b)(2).

Attorney Docket No.	624.P	Type a plus sign (+) inside this box ----->	+

INVENTOR(s)/APPLICANT(s)

LAST NAME	FIRST NAME	MIDDLE INITIAL	RESIDENCE (CITY AND EITHER STATE OR FOREIGN COUNTRY)
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22386 U.S. PTO 60/590987 072604

TITLE OF THE INVENTION (280 characters max)

HPV INHIBITORS

CORRESPONDENCE ADDRESS

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Foster City

STATE	California	ZIP CODE	94404	COUNTRY	U.S.A.

ENCLOSED APPLICATION PARTS (check all that apply)

- [X] Specification — *Number of pages* 19
- [ ] Small Entity Statement
- [ ] Drawing(s) — *Number of sheets*
- [ ] Other (specify) _____

METHOD OF PAYMENT (check one)

- [X] The Commissioner is hereby authorized to charge filing fees (as well as any additional fees which may be required by this paper) and credit Deposit Account Number **07-1250**. Provisional Filing Fee Amount ($) **$ 160.00**

The invention was made by an agency of the United States Government of under a contract with an agency of the United States Government.

- [X] No.
- [ ] Yes, the name of the U.S. Government Agency and the Government contract number are:

Respectfully submitted,

SIGNATURE James J. Wong DATE July 26, 2004

TYPED or PRINTED NAME James J. Wong REGISTRATION NO. (if appropriate) 34,949

- [ ] Additional inventors are being named on separately numbered sheets attached hereto

PATENT

Attorney Docket No. 624.P

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Jianying Wang

For: HPV Inhibitors

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

PROVISIONAL APPLICATION COVER SHEET
(37 C.F.R. § 1.51 (2) (i))

CERTIFICATION UNDER 37 CFR 1.10

I hereby certify that this "Provisional Application Cover Sheet" and the documents referred to as attached therein are being deposited with the United States Postal Service on this date July 26, 2004 in an envelope as "Express Mail Post Office to Addressee" Mailing Label Number EV 382658112US addressed to the: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

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HPV INHIBITORS

Most Prevalent Serious HPV-Mediated Diseases (US and EU)

- Anal dysplasia in HIV patients
  - low & high-grade dysplasias ~ 575,000
- Cervical dysplasia
  - high-grade lesions ~ 470,000
  - low-grade ~ 12MM
- Genital warts
  - ~2.3MM
  - 280,000 patients/yr receive topical pharmaceutical therapies
- Anal dysplasia in homosexual males
  - low & high-grade dysplasias ~ 700,000

Why is Gilead Interested in HPV

- Series of nucleotides have in vitro activity in HPV+ cell lines
- Cidofovir and analogs effective in animal models
  - SiHa xenograft and CRPV models
- Cidofovir has shown some efficacy in HPV associated human diseases
  - anogenital warts, cervical intraepithelial neoplasia, respiratory papillomatosis
- HPV-associated diseases are an unmet medical need
- Some indications, particularly anal dysplasia in HIV patients, are a good corporate fit

Human Papillomaviruses

- Small, non-enveloped, DNA virus
- Dependent on host cell for replication
- Epithelial tropism
  - site of occurrence (cutaneous, mucosal)
- Species specific with > 100 human subtypes
  - low and high risk subtypes

HPV Genotypes

- High risk:
  - HPV-16, 18, 31, 33, 35, 45
  - potential to induce malignant proliferation
    - HPV-16,18 responsible for 50-80% of dysplasias
    - untreated dysplasias may develop into cancer
- Low risk:
  - HPV-6, 11
  - responsible for nearly 90% of genital warts

Target Product Profile Overview

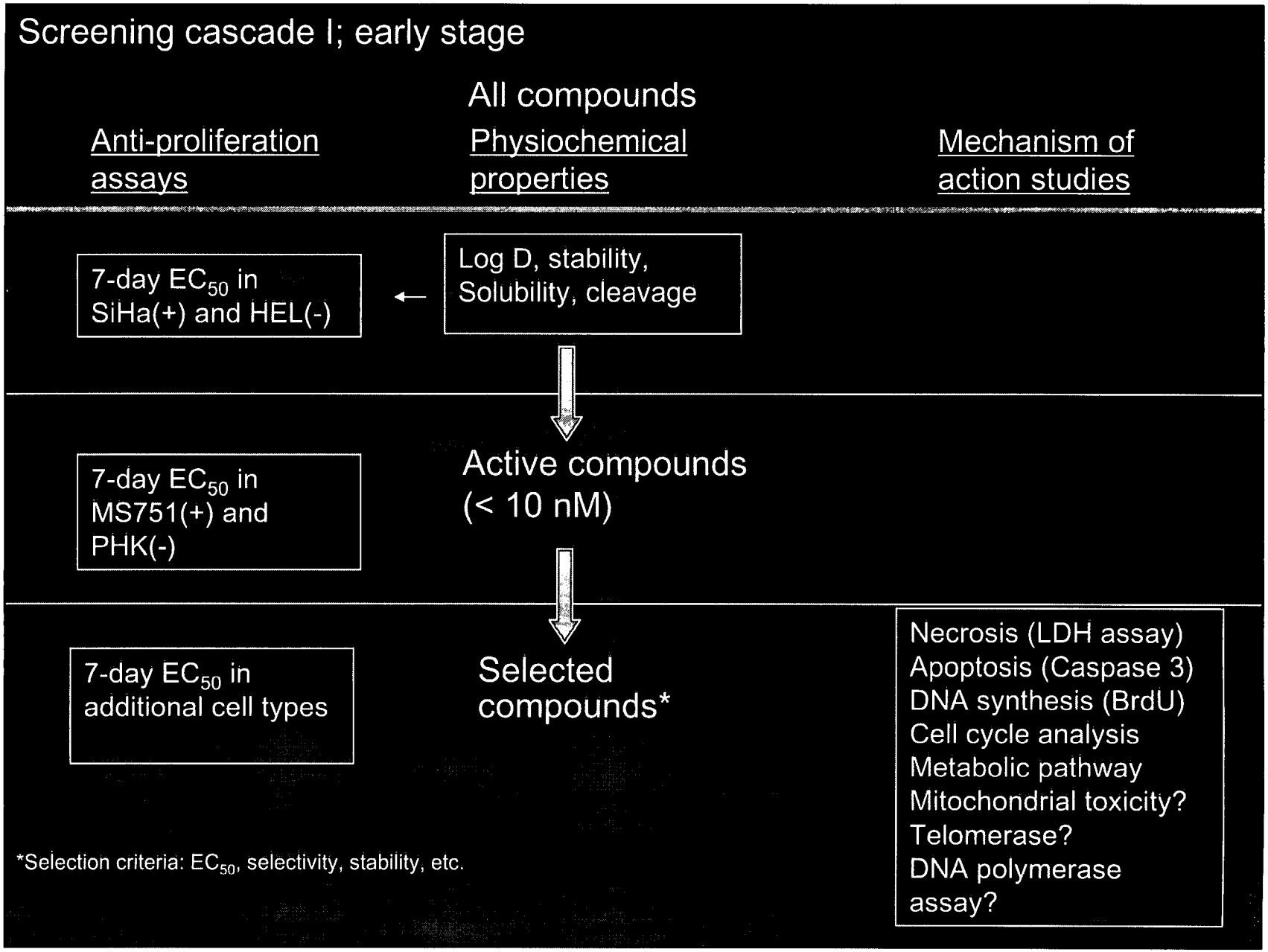
- HPV Activity
  - activity against HPV-16,18; ideally activity against HPV-6,11
- Selectivity
  - good selectivity between HPV-infected and non-infected tissue
- Safety
  - minimally irritating to mucosal tissue
  - non-mutagenic
  - ideally, non-teratogenic
- Dosing
  - once-daily dosing acceptable for anal and cervical dysplasias and for genital warts
- Formulation
  - topical gel/foam/cream

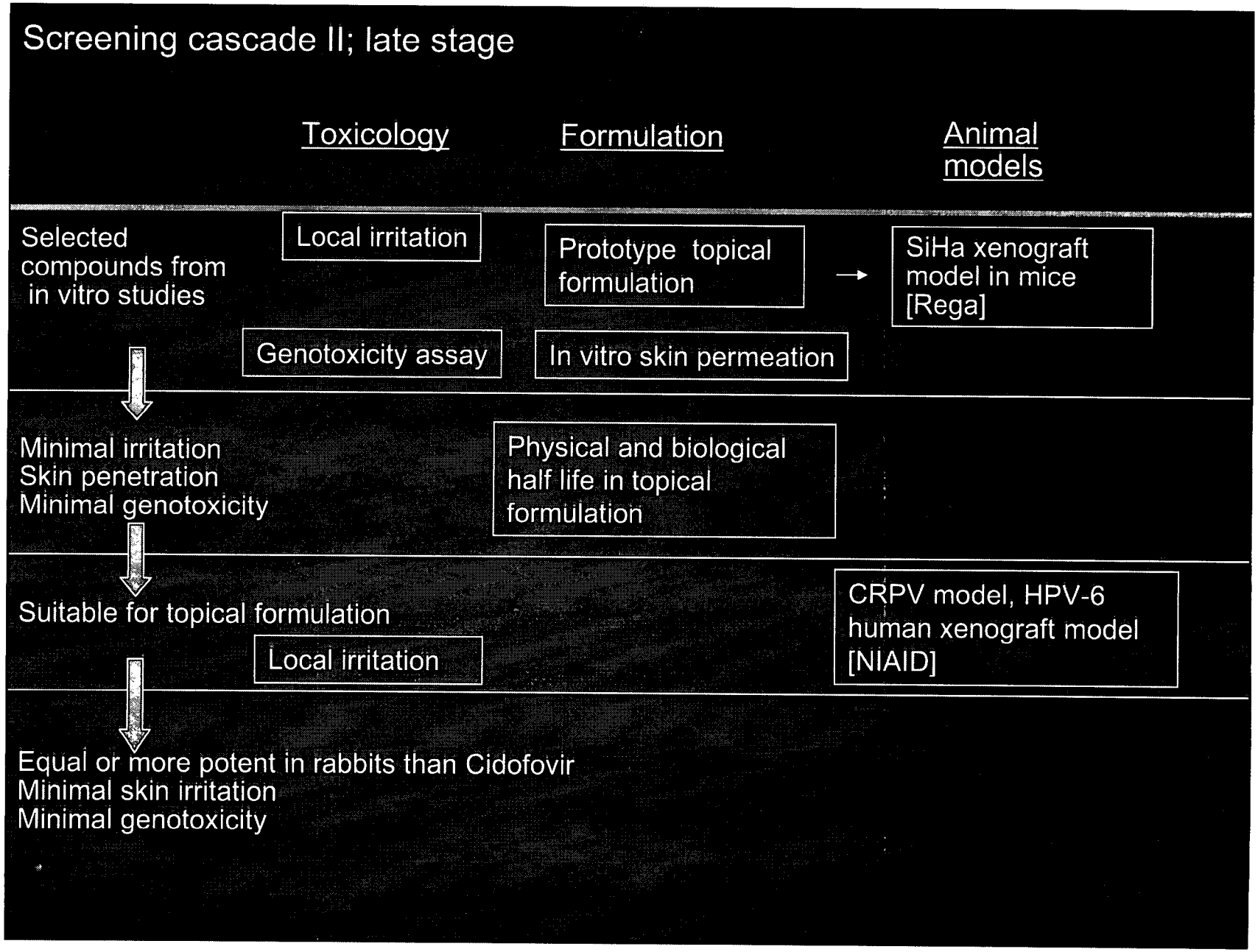
Goals for Program

- Topical prodrugs
  - improve potency
  - allow for skin penetration
  - reduce toxicity
- Selectivity in vitro
  - EC_{50} "normal" cell line/EC_{50} HPV+ cell line
- Topical efficacy in representative animal models
- Compound with minimal irritation and minimal/no genotoxicity

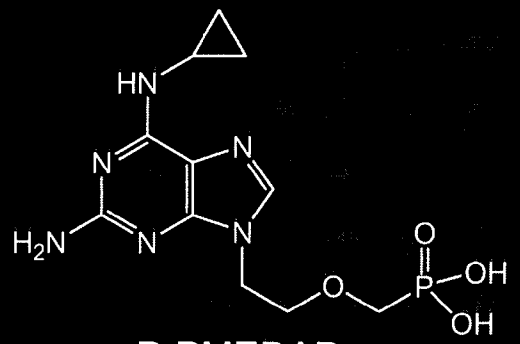
Key Challenges

- Selectivity index is low in vitro
- Mechanism of action is not understood
- The parent molecules are potentially toxic
  - renal toxicity, mutagenicity, carcinogenicity, local irritation


Screening cascade I; early stage
All compounds
Anti-proliferation assays
Physiochemical properties
Mechanism of action studies
7-day EC50 in SiHa(+) and HEL(-)
Log D, stability, Solubility, cleavage
Active compounds (< 10 nM)
7-day EC50 in MS751(+) and PHK(-)
Selected compounds*
7-day EC50 in additional cell types
Necrosis (LDH assay)
Apoptosis (Caspase 3)
DNA synthesis (BrdU)
Cell cycle analysis
Metabolic pathway
Mitochondrial toxicity?
Telomerase?
DNA polymerase assay?
*Selection criteria: EC50, selectivity, stability, etc.


Screening cascade II; late stage
Toxicology
Formulation
Animal models
Selected compounds from in vitro studies
Local irritation
Prototype topical formulation
SiHa xenograft model in mice [Rega]
Genotoxicity assay
In vitro skin permeation
Minimal irritation
Skin penetration
Minimal genotoxicity
Physical and biological half life in topical formulation
Suitable for topical formulation
CRPV model, HPV-6 human xenograft model [NIAID]
Local irritation
Equal or more potent in rabbits than Cidofovir
Minimal skin irritation
Minimal genotoxicity

cPrPMEDAP: Scaffold for Prodrug Design



cPrPMEDAP

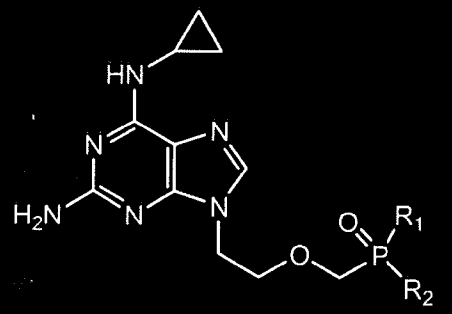
- Anti-proliferative activity against HPV(+) cell lines (EC_{50} at sub-μM range)
- Selectivity when compared to HPV(-) cell lines or primary human keratinocytes

Disadvantages of Other Parent Scaffolds

- Cidofovir (HPMPC) has moderate activity but less selective
- PMEA is not active
- PMEDAP and its other analogs are less active
- PMEG has similar activity; synthetically more challenging

Improved Potency of cPrPMEDAP Prodrugs

GS#	R1	R2	EC_{50} (nM) in HPV16+ SiHa cell line
8369	OH	OH	284
17429	O-iPr	O-iPr	2267
327353	Ala-Pr	Ala-Pr	2.5
327238	Ala-iPr	Ala-iPr	1.3
327319	Aba-Et	Aba-Et	3.2
327261	Aba-Bu	Aba-Bu	0.20
327352	OPh	Ala-Pr	0.50
327383	OPh	Aba-Bu	0.13
56884	OPh	Phe-Et	0.60



8369 (cPrPMEDAP)

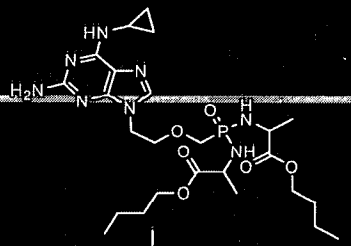
Selection Criteria for Initial *in vivo* Screens

- Key criteria
  - Selectivity HPV+/HPV- cell lines
  - Log D between 1.5 and 2.5
  - Stability in formulation vehicle
- Other
  - Potency in HPV+ cell lines
  - Solubility
  - Cleavage by CAT A

cPrPMEDAP Prodrugs with Good Selectivity

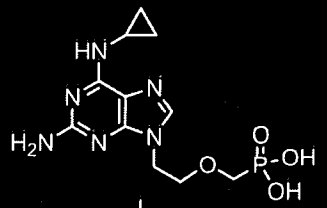
GS#	Structure		Selectivity	
	R1	R2	HEL/SiHa	PHK/SiHa
8369	OH	OH	17	13
327353	Ala-Pr	Ala-Pr	210	31
327238	Ala-iPr	Ala-iPr	559	75
327319	Aba-Et	Aba-Et	135	12
327261	Aba-Bu	Aba-Bu	115	4
327352	OPh	Ala-Pr	164	10
327383	OPh	Aba-Bu	92	22
56884	OPh	Phe-Et	72	58
Podofilox	Active ingredient of condylox		<0.9	0.1
8358	PMEG bis Ala-Bu		11	1.8
AraC	C-analog DNA pol inh		0.11	0.57
Cladribine	A-analog DNA pol inh		nd	1

Mechanism of Selectivity (hypothesis)



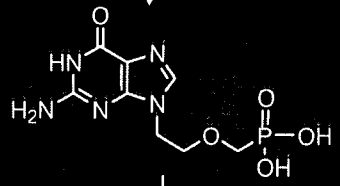
cprPMEDAP amidate

Hydrolase (CatA?)



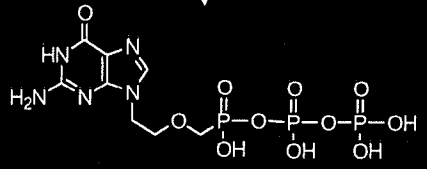
cprPMEDAP

Deaminase?



PMEG

Kinases



PMEGpp
=active molecule

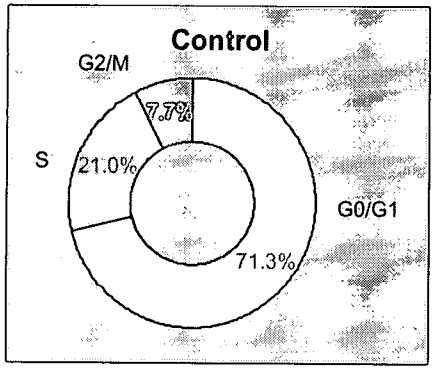
Some of these reactions might be more active in HPV transformed cells than in normal cells

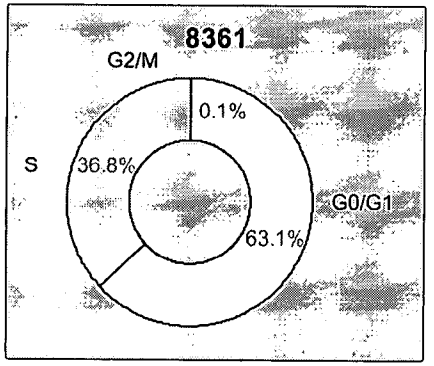
Biological effect

1. **Pol α/δ inhibition**
2. **Cell cycle arrest at the S phase**
3. **Induction of apoptosis**

A prototype prodrug arrests SiHa cells at the S-phase of the cell cycle and induces apoptosis

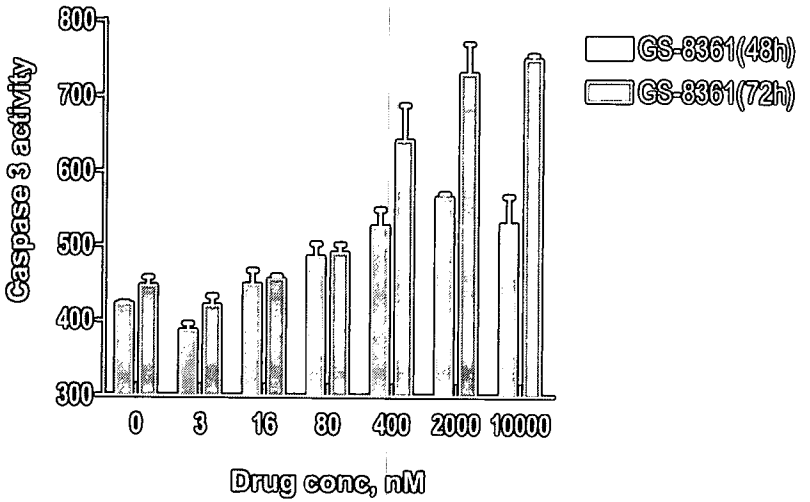
Cell cycle analysis
48 hr

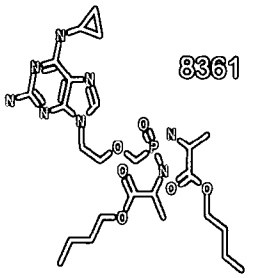




4 nM 8361 (10 fold EC50)

Induction of Apoptosis

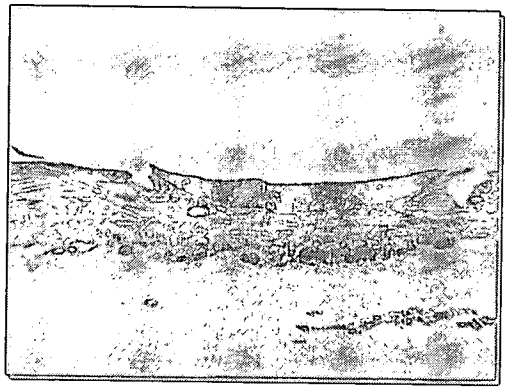




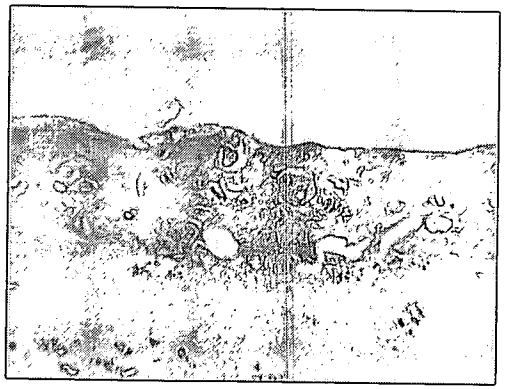
Mechanism of Selectivity
(experimental plan)

- Compare the rate of metabolic conversion in cells to identify the rate-limiting reaction
  - SiHa (HPV16, sensitive to cprPMEDAP amidates)
  - CaSki (HPV16, resistant)
  - Primary keratinocytes (HPV neg, somewhat resistant)
  - Primary fibroblasts (HPV neg, resistant)

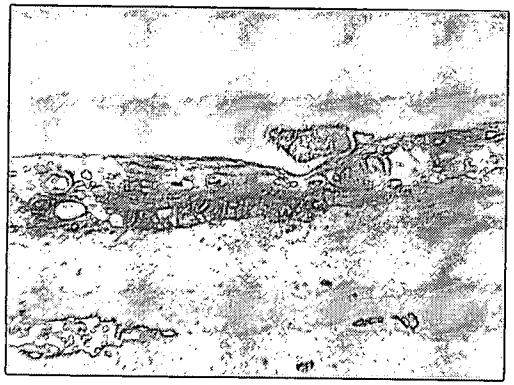
PHK-SiHa Raft Co-cultures
(after 10 days of differentiation)



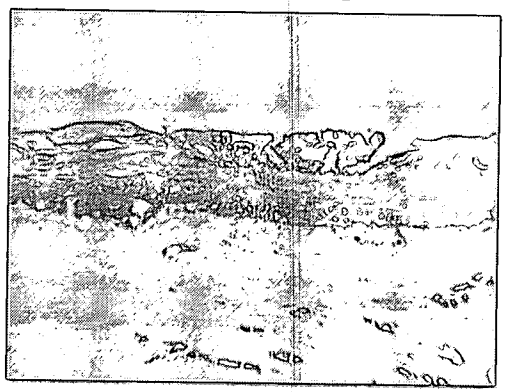
Control



cPrPMEDAP 5 µg/mL

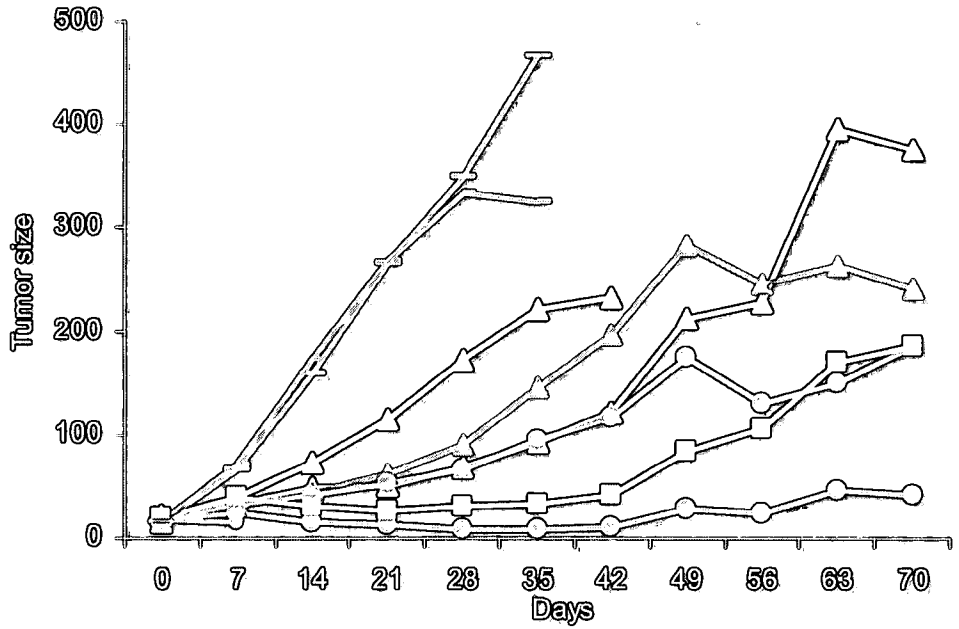


cPrPMEDAP 0.5 µg/mL



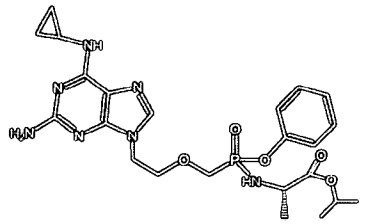
cPrPMEDAP 0.05 µg/mL

SiHa Tumor Model - Athymic Mice
Monoamidates (Ala-iPr)



77384 0.5mg/ml
77384 0.1mg/ml
77384 0.05mg/ml
17432 5mg/ml
17432 1mg/ml
17432 0.5mg/ml
CDV 10mg/ml
PBS
Control

3 weeks treatment, 5 days/week
100 µl, intratumoral



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